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Comparison of the acute chromic capacities of erythropoietin and U-74389G concerning mean corpuscular hemoglobin concentration levels

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ANALYSIS ARTICLE

ABSTRACT

Aim: This study compared the chromic capacities of erythropoietin (Epo) and antioxidant drug U-74389G based on 2 preliminary studies. The provided results at mean corpuscular hemoglobin concentration (MCHC) levels alterations were co-evaluated in a hypoxia reoxygenation protocol of an animalmodel.

Materials and methods: MCHC levels (MCHCl) were evaluated at the 60threoxygenationmin (for groups A, C and E) and at the 120threoxygenationmin (for groups B, D and F) in 60 rats. Groups A and B received no drugs, rats from groups C andD were administered with Epo; whereas rats from groups Eand Fwere administered with U-74389G.

Results: The first preliminary study of Epo presented a significant hyperchromic capacity of the MCHCl by 0.89%±0.31% (p-value=0.0061). However, the second preliminary study of U-74389G presented a non significant hypochromic capacity of the MCHCl by 0.69%±0.37% (p-value=0.0655).These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that U-74389G has opposite chromic potency than Epo (p-value=0.0000).

Conclusions: The anti-oxidant capacities of U-74389G has hypochromic capacity than epo which presents hyperchromic capacity (p-value=0.0000).

Key words: hypoxia; erythropoietin; U-74389G; mean corpuscular hemoglobin concentration ..vbslevels; reoxygenation

1. INTRODUCTION

The acute hypochromic capacity[1] of U-74389G is thus not significant (p-value=0.0655).U-74389G is a novel antioxidant factor. It implicates just only 255 known biomedical studies at present. 4.31% of these studies concern tissue hypoxia and reoxygenation (HR) experiments. The promising effect of U-74389G in tissue protection has been noted in these HR studies. U-74389G or also known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation. It protects against HR injury in animal heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers. Lazaroids, a novel series of glucocorticoid compounds 21-aminosteroids have the properties of free radical scavenging. U-74389G is one of the 132 similar lazaroid compounds. It has a molecular weight of 726.90406 g/mol; it has a selective action on vascular endothelium with vitamin E-like properties.

However, the hypochromic capacity of U-74389G gets more comprehensible whether is compared with the same capacity of a standard known drug. Such one of the most well studied drug; actually with original erythropoietic capacity (p-value=0.0061) is erythropoietin (Epo). Indeed, Epo implicates over 29,447 known biomedical studies at present. 10.44% at least of these studies concern tissue hypoxia and reoxygenation (HR) experiments. Certainly, the concept has been moved away from the original action of Epo in stem blood cells recovery. However, just few related reports were found, not covering completely the specific matter with antioxidant factors.

The special aim of this experimental work was to compare the acute chromic capacities of U-74389G and Epo on a rat model and mainly in an HR protocol. Their effects were tested by measuring the mean corpuscular hemoglobin concentration (MCHC) levels.

2. MATERIALS AND METHODS

Animal preparation

The Vet licenses of the research were provided under 3693/12-11- 2010 & 14/10-1-2012 decisions. The granting company and the place of experiment are mentioned in related references[1,2]. Appropriate humanistic care were adopted for Albino female Wistar rats.

7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Continuous intra-experimental general anesthesia, oxygen supply, electrocardiogram, acidometry and post-experimental euthanasia were provided. Rats 16 – 18 weeks old were randomly delivered to six (6) groups (n=10), using the following protocols of HR: Hypoxia for 45 min followed by reoxygenation for 60 min (group A); hypoxia for 45 min followed by reoxygenation for 120 min (group B); hypoxia for 45 min followed by immediate Epo intravenous (IV) administration and reoxygenation for 60 min (group C); hypoxia for 45 min followed by immediate Epo IV administration and reoxygenation for 120 min (group D); hypoxia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group E); hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group F). The dose height selection criteria of Epo and U-74389G were assessed at preliminary studies as 10 mg/Kg body mass of animals for both drugs.

Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior aorta patency and reoxygenation. After exclusion of the blood flow, the protocol of HR was applied, as described above for each experimental group. The drugs were administered at the time of reperfusion; through inferior vena cava catheter. The MCHC levels (MCHCl) were determined at 60th min of reoxygenation (for A, C and E groups) and at 120th min of reoxygenation (for B, D and F groups).The animals' mass was not a confusing factor for MCHCl presenting a non significant relation (p-value=0.1378).

Statistical analysis

Table 1 presents the (%) augmentation influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) reduction influence of U-74389G regarding reoxygenation time. Chi-square tests were applied using the ratios which produced the (%) results per endpoint. The outcomes of chi-square tests are depicted at Table 3. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

3. RESULTS

The successive application of chi-square tests revealed that U-74389G reduced the MCHCl than Epo which increased them by -.2774225-fold [-.2769503 - -.2778955] at 1h, by -.5504722-fold [-.549719 - -.5512263] at 1.5h, by -.8522433-fold [-.8511911 - -.8532967] at 2h, by 3.044774-fold [3.035879 - 3.053694] without drugs and by -.7793243-fold [-.7779941 - -.7806567] whether all variables have been considered (p-value=0.0000).

Table 1 The (%) augmentation influence of erythropoietin in connection with reoxygenation time

Augmentation	***±SD***	***Reoxygenation time***	***p-value***
1.82%	±1.85%	1h	0.0076
1.73%	±2.18%	1.5h	0.0016
1.65%	±2.57%	2h	0.0721
-0.17%	±2.48%	reperfusion time	0.7555
0.90%	±0.32%	interaction	0.0061

Table 2 The (%) reduction influence of U-74389G in connection with reoxygenation time

Reduction	***±SD***	***Reoxygenation time***	***p-values***
0.50%	±2.22%	1h	0.4820
0.95%	±2.41%	1.5h	0.1124
1.40%	±2.62%	2h	0.1603
0.53%	±2.73%	reperfusion time	0.3955
0.70%	±0.38%	interaction	0.0655

Table 3 The U-74389G / erythropoietin efficacies ratios on MCV levels augmentation after chi-square tests application

Odds ratio	*[95% Conf. Interval]*		*p-values*	*Endpoint*
-.2774225	-.2769503	-.2778955	0.0000	1h
-.5504722	-.549719	-.5512263	0.0000	1.5h
-.8522433	-.8511911	-.8532967	0.0000	2h
+3.044774	+3.035879	+3.053694	0.0000	reperfusion time
-.7793243	-.7779941	-.7806567	0.0000	interaction

Table 4 A U-74389G / erythropoietin efficacies ratios meta-analysis on 3 seric variables[12]

Endpoint / Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
Hematocrit	38.424	0.0000	9.076658	0.0000	6.222898	0.0000	1.001356	0.2184	12.66419	0.0000
Hemoglobin	1.268689	0.0000	1.839035	0.0000	13.1658	0.0000	1.252422	0.0000	1.94889	0.0000
Creatinine	168.9034	0.0000	4.872332	0.0000	3.039572	0.0000	1.0262016	0.0000	5.005523	0.0000
Mean	20.1929009	0.0000	4.33262345	0.0000	6.29145057	0.0000	1.08773713	0.0728	4.98048231	0.0000

4. DISCUSSION

The unique available study investigating the declining effect of U-74389G on MCHCl was the preliminary one[1]. Although the most famous activities of neuroprotection and membrane-stabilization properties, it accumulates in the cell membrane, protecting vascular endothelium from peroxidative damage but hardly penetrates the blood-brain barrier. It elicits a beneficial effect in ototoxicity and Duchenne muscular dystrophy. It increases γGT, SOD, and GSH levels in oxygen-exposed cells. It treats septic states and acts as immunosuppressant in flap survival. It prevents the learning impairments; it delays the early synaptic transmission decay during hypoxia improving energetic state of neurons. It shows antiproliferative properties on brain cancer cells and is considered as a new promising anti inflammatory drug for the treatment of reperfusion syndrome in IR injuries.

The same authors confirmed[2] the short-term hyperchromic effect of Epo preparations in non iron deficient individuals. Yuan JQ et al compared[3] the incidences of anemia, after the application of different endocrine therapies in patients with prostate cancer. After 12 months, Hb (P=0.016), in hormone group were significantly lower than in the castration group (P=0.006). The endocrine therapies can be associated with anemia in patients with prostate cancer. Chu HC et al described[4]reelin as an extracellular glycoprotein, present in erythroid cells that is up-regulated during erythroid differentiation of human erythroleukemic K562 cells. Reelin deficiency promotes erythroid differentiation of K562 cells; however its deficiency attenuates AKT phosphorylation of the Ter119(+)CD71(+) erythroid progenitors and alters the cell number and frequency of the progenitors at different erythroid differentiation stages. A regulatory role of Reelin in erythroid differentiation is thus defined. Ribeiro IF et al observed[5] significant responses of erythropoietin (EPO T→G) polymorphism for the EPO TT genotype in mean corpuscular hemoglobin concentration values in runners before and after 14 days of 400 mg pequi oil supplementation. Miller BA described TRPC2 as[6] a pseudogene in humans. Its activation by DAG and by erythropoietin and its inhibition byCa(2+)-calmodulin are known. The red cells of TRPC2 knockout mice showed reduced mean corpuscular hemoglobin concentration. TRPC2-depleted red cells were resistant to oxidative stress-induced hemolysis. Kaliev R et al proved[7]that Epoetin beta used in patients with chronic glomerulonephritis has an anti-anemic effect combined with hypoxic altitude chamber training (HACT); since there were increases in the concentration of MCHC in the patients receiving EPO ($p<0.05$) over time. Ito C et al found the ratio of MCH in group continuous erythropoiesis receptor activator (CERA) decreased compared with that in group darbepoetin alfa (DA) in non-dialysis chronic kidney disease (CKD) patients. Reticulocyte indices could provide a more detailed explanation. Zubrikhina GN et al found[9] hypochromic anemia (mean corpuscular hemoglobin concentration, <27.0 pg) in true iron deficiency (ID). The EPO level corresponded to the degree of anemia and was 4-5-fold the normal values. In anemia of chronic diseases (ACD)s, 45% of patients had hypochromic anemia and that of EPO

corresponded to the degree of anemia in 26% of the patients. Anemia in Hodgkin's and non-Hodgkin's lymphomas was also hypochromic. The pharmacokinetic parameters including EPO should be determined for the differential diagnosis of true and functional ID. Sharma JB et al correlated[10] the serum erythropoietin levels with other hematological parameters in normal pregnant and anemic pregnant patients. MCHC was reduced in anemic pregnancies than non-anemic ones by 11.69% (p=0.176), serum erythropoietin levels were significantly higher in anemic women by 44.81% (p=0.064). The levels were significantly higher in severe anemia by 156.62%, than in moderate anemia by 51.21% and mild anemia by 25.82%. Various hematological parameters including serum erythropoietin levels correlate with the severity of anemia.

Haddad J Jr et al examined[10] the effect of intraperitoneal injections of 40 mg/kg of the lazaroid compound U-74389G every 12 hours, on acute otitis media induced by Streptococcus pneumoniae organisms inoculated into the right tympanic cavity; with the left ear served as a control one in guinea pigs.

According to above, table 3 shows that U-74389G attenuated by -.7793243-fold [-.7779941 - -.7806567] the MCHCl than Epo (p-value=0.0000); a trend accentuated along time, in Epo non-deficient rats. However,a meta-analysis of these ratios from the same experiment, for 3 other seric variables, provides opposite results (table 4).

5. CONCLUSION

The anti-oxidant capacities of U-74389G attenuated by -.7793243-fold [-.7779941 - -.7806567] the MCHCl than Epo (p-value=0.0000) in rats. However, this trend is accentuated along the short term time frame of the experiment.

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